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Note added in proof

In a redent study, Licht *et al.* [111] identified only cGnRH-II and mGnRH in the brain of three species of *Rana* (*pipiens, ridibunda* and *esculenta*). While both GnRHs were distributed in the telencephalon and diencephalon, only cGnRH-II was found in the cerebellum and medulla. In the platyfish, lGnRH, together with mGnRH and sGnRH was revealed by ICC in some cells of the pituitary gland of animals of all ages [112].

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Effects of Rapid Cooling on Heart Rate of the Japanese Lobster *in vivo*

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ABSTRACT—Seasonal changes of the effects of rapid cooling on the *in vivo* heart rate of the lobster, *Panulirus japonicus*, were studied. Cardiac activity was monitored with chronically implanted electrodes. The mean heart rate was 45.5±6.0 bts/min during winter and 99.5±7.5 bts/min during the other seasons at the same acclimation temperature (20±1°C). When the ambient temperature was lowered (0.1–1.0°C/min, dT=5-6°C), the heart rate decreased along a linear line in spring, summer and fall. The correlation ratio of the heart rate and temperature was 0.98 with the minimum rate of 67.0 ±12.0 bts/min. The Q_{10} value was 2.2. In contrast, the heart rate in winter decreased only for the initial few minutes of cooling and little for the later phase with the minimum rate of 28.8±9.5 bts/min. Q_{10} was 2.6. The correlation plots for the heart rate and temperature appeared to regress on two linear lines. The ratio was 0.88 in the range of 19–21°C and 0.41 in the range of 15–19°C. These data may suggest that the lobsters in winter have some compensatory mechanisms for a heart rate drop by cooling as well as for the mean heart rate, which are different from those in the other seasons.

INTRODUCTION

The heart rate of crustaceans changes with variations in ambient temperature within the normal environmental range. Temperature coefficient (Q_{10}) for the range of 10–20°C of the crustacean heart rate is around 2, measured *in vivo* for slow temperature changes ([24] for review, [10]). Even though marine lobsters live under mild temperature environment, they meet daily with a warm or cold water brought about by tidal cycle. Moreover, they suffer seasonal changes. Our preliminary experiments show that temperature of the lobster pericardium will follow the change in ambient temperature within 30 sec. Electromechanical coupling of muscle fibers becomes less efficient with decreasing temperature [8]. A compensation mechanism has been described for leg muscle activity of crabs [9]. It may be that the lobsters are equipped with compensation mechanisms in the heart activity for a rapid drop in temperature. Few studies on the heart response to rapid temperature changes in crustaceans have been reported [28]. However, the presence of compensation in heart rate has been still unclear.

In the present study, the influence of rapid cooling on heart rate *in vivo* is examined over all seasons using Japanese spiny lobsters. The behavior of heart rate in winter was different from that in the other seasons.

MATERIALS AND METHODS

Japanese spiny lobsters (*Panulirus japonicus*, around 200g in body weight, both sexes, n=8) were used. They were captured around Nabeta Bay (Izu Peninsula, Japan) in April and October and were reared in an indoor aquarium ($1.5\times4\times1m^3$) where the fresh natural sea water was continuously supplied. Natural light (around 20k lx at noon) was provided *via* windows. Electrocardiogram (ECG) was recorded from the unrestrained animals with chronically implanted electrodes. The recording methods were similar to those described previously [13, 14]. The electrodes consisted of silver wires, which were introduced through small holes drilled in the carapace of the dorsal thorax and fixed in position with epoxy resin. Then each lobster with the electrodes mounted was kept in a tank ($25\times40\times30$ cm^3) for a month before being subjected to cooling experiment. They were fed on a diet of live *Mytilus*. Coral sands were laid on the bottom of the tank as suggested by Florey and Kriebel [10]. The sea water in the tank was aerated and stirred with air and water pumps, respectively. The acclimation temperature was 20± 1°C because seasonal changes of the sea water temperature in the aquarium have ranged from 15 to 25°C. To cool the animal, the sea water cooled at 5°C was poured into the tank and mixed by stirring. Water temperature was monitored with an electronic thermometer. The cooling rates (0.1–1.3°C/min) and magnitudes (dT=5–6°C) were determined by pouring rate and volumes of the cold sea water. During experiment, major part of the tank was covered with black screen to minimize disturbance. Electrical signals amplified were recorded on a video tape (DC-2K Hz). These measurements were performed once a week. The electrical recordings were played back to a pen recorder (100 Hz). The heart rate was counted by hand and/or with an electronic counter using pulses of ECG. Statistical analysis of the data was performed with a personal computer software (StatView II: Abacus Concepts, Inc.).

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RESULTS

Beating patterns of the heart became constant at 2 weeks after the lobster was transferred into the experimental tank. Thus the acclimation period of a month was long enough for the lobster to adapt to the experimental conditions. Large variations of heart rate accompanying with the body movement and spontaneous bradycardia often occurred. However, it was possible to measure the rate in a stable state since the animal often kept quiescent for 10 or more minutes in daytime. Complete recordings of ECG *in vivo* were obtained frequently during cooling. However, only few recordings were reliable for both cooling and rewarming processes because the unrestrained lobsters moved vigorously